

# DNA templates and plasmids

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 An abbreviated version of this protocol was published in eLIFE in Apr 2021

NusG is an intrinsic transcription termination factor that stimulates motility and coordinates gene expression with NusA

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## Detailed protocol

PCR reagent final concentrations (50 uL total per reaction):

10X ThermoPol Reaction Buffer - 1X (in this case we put 5 uL in to the reaction)

dNTPs - 0.75 mM

F primer - 2 uM

R primer - 2 uM

template (pAY196) - ~4 ng/uL

additional MgSO<sub>4</sub> - 2 mM (1 uL from 100 mM stock, which typically comes with Vent Pol)

Vent DNA Polymerase (NEB, cat # M0254S) - 2 units

H<sub>2</sub>O - mark up to 50 uL

25 cycles of:

initial denaturation - yes

denaturing temp - 94 °C - 30 seconds

annealing temp - 55 °C - 30 seconds

extension temp - 72 °C - 3 minutes

final extension - yes

check that PCR worked on a 0.8% agarose gel with 1 kb DNA ladder for reference. look for band ~2862 bp in length.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Mandell, Z. and Babitzke, P. (2022). DNA templates and plasmids. Bio-protocol Preprint. [bio-protocol.org/prep1495](https://doi.org/10.21203/rs.3.rs-1149514/v1).
2. Mandell, Z. F., Oshiro, R. T., Yakhnin, A. V., Vishwakarma, R., Kashlev, M., Kearns, D. B. and Babitzke, P. (2021). NusG is an intrinsic transcription termination factor that stimulates motility and coordinates gene expression with NusA. eLIFE. DOI: [10.7554/eLife.61880](https://doi.org/10.7554/eLife.61880)

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